Docket No.: 1997748US0PCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:

Michinobu NAKAMURA, et al.

SERIAL NO.: 09/674,280

FILED: DECEMBER 21, 2000

FOR: PROCESS FOR PRODUCING PROTEIN HYDROLYZATE

: EXAMINER: AFREMOVA

: GROUP ART UNIT: 1651

DECLARATION UNDER 37 C.F.R. §1.132

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Now comes Toshimasa Ishii, who deposes and states that:

1. That I am a graduate of Hokkaido University, Japan, and received my Master's degree in microbiology, in the year 1989.

2. That I work as a researcher for Ajinomoto Co., Inc., the assignee of the above-identified application by virtue of the Assignment of Application submitted herewith, since graduation in the year 1989.

3. That I understand the English language or, at least, that the contents of the Declaration were made clear to me prior to executing the same,

4. That I have reviewed the following:

A. The specification and claims of U.S. Patent Application Serial No. 09/674,280 ("the '280 application") and all amendments thereof;

B. The Official Action dated October 21, 2002, in the '280 application;

U.S. Patent No. 4,808,419 (Hsu);

U.S. Patent No. 5,888,561 (Niederberger et al);

E. U.S. Patent No. 6,045,819 (Takebe);

F. WO 95/28853 (Muller et al).

5. That Figure 3 of the '280 application reveals that glucose content increases with an increase in the reaction temperature and with a lapse in the reaction time. When the temperature was set at 45 ℃, glucose is highly produced and is not being decomposed, in contrast to setting the temperature at 36 ℃. In general, the temperature is set around 45°C, as long as the hydrolysis is conducted by using *Aspergillus oryzae*, which is the same microorganism employed in carrying out experiments of the '280 application. The reason that the temperature at 45°C is preferred is because it is suitable for hydrolyzing polysaccharides to produce large amounts of glucose. However, the glucose so produced can be material for "browning reaction," which is a problem that the '280 application seeks to avoid (see pages 5-6).

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6. That Figure 2 of the '280 application reveals that the glutamic acid content increases with an increase in the reaction temperature and with a lapse in the reaction time. Further, it is clear that the rate of production of glutamic acid at 45°C is quicker than that at 36°C. However, the objects of the '280 application can only be obtained if the hydrolysis speed can be maintained and the "browning reaction" can be prevented.

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7. That two different microorganisms are usually employed in order to fulfill the objects of the '280 application. In many procedures, including Hsu et al, one bacterium (such as *Aspergillus oryzae*) is employed to hydrolyze polysaccharides to produce glucose, while a second microoganism (such as yeast or another suitable microorganism) is employed to produce ethanol or acetic acid from glucose.

8. That the '280 application does not change microorganism throughout the entire process. As is evident in the Examples of the '280 application only *Aspergillus oryzae* is employed to satisfy the objects of the present invention (see pages 22-32). However, the

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difficulties mentioned above were still encounterd with only using *Aspergillus oryzae* since it was cultivated under the same condition.

9. That the Inventors of the '280 application surprisingly solved the problems described for *Aspergillus oryzae* by introducing "temperature shift." Specifically, the Inventors of the '280 application successfully solved the problems associated with the commonly employed methods by shifting the temperature from lower temperature to higher temperature. Moreover, by adding the temperature shift into the process, the Inventors have achieved their goal of 1) maintaining hydrolysis speed and 2) hindering "browning reaction" due to the consumption of sugar.

10. That the following experiment was carried out by me or under my direct supervision and control, and show that the method of the '280 application, which employs a "temperature shift," results in an unexpected advantage of preventing the browning reaction by reducing the sugar content.

11. That the protocol for the experiment is as follows;

(a) Production of wheat gluten dispersion and sterilization

City water at 90 °C was charged into a dispersion vessel fitted with a stirrer having a high stirring power. Wheat gluten was added thereto at a concentration of 50 g/l. These contents were stirred and mixed for 1 hour. The resulting dispersion was heat-sterilized at a temperature between 120 and 140 °C.

(b) Preparation of a liquid koji culture

A culture medium obtained by dispersing de-fatted soybean powder to 15 g/l was heat-sterilized at a temperature between 120 and 140 °C. A liquid seed culture of a koji mold, *Aspergillus oryzae*, grown from isolated spores in an aseptic culture medium in whichh de-fatted soybean powder had been dispersed at a concentration of 15 g/l, was inoculated in

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the aforementioned heat-stearlized culture medium in an amount of 15%. After inoculation thereof, incubation was performed with aeration and stirring at 30 °C for 24 hours to obtain a koji mold culture.

(c) Hydrolysis of wheat gluten dispersion

A sterilized gluten dispersion was introduced into a sterilized fermenter. The liquid koji mold culture prepared in (b) was added in one-forth of the wheat gluten dispersion. The enzymatic hydrolysis was performed with aeration and stirring for 24 hours.

TOTAL 21 24hrs →

one-third

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The temperature conditions and corresponding hydrolysis rates were determined to be as described in Table A below:

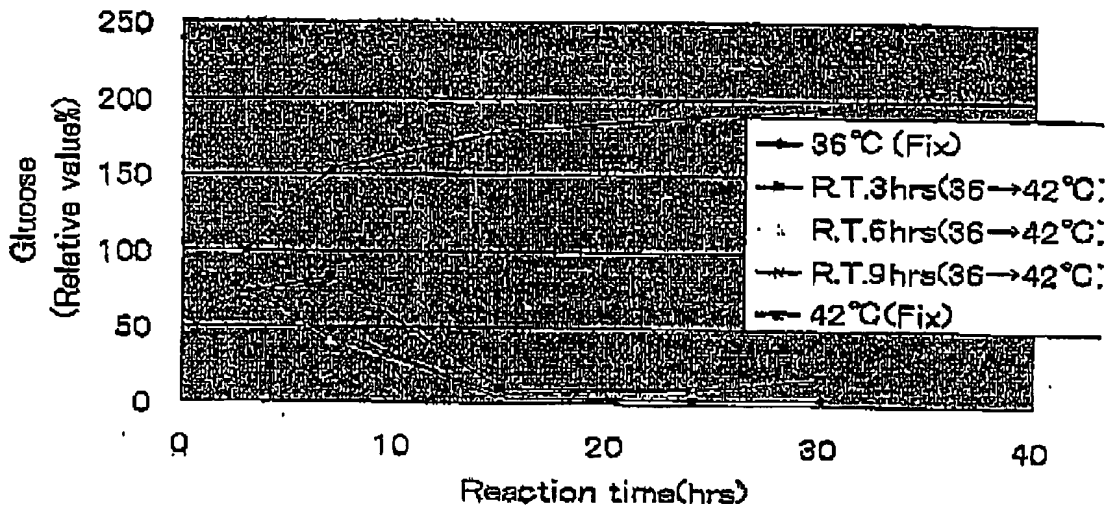
Table A Effect of Temperature on Hydrolysis rate

Temperature (°C)	Hydrolysis rate (Relative value%)
36°C (Fixed)	68
Temperature shift at 3 hours from 36 to 45°C	108
Temperature shift at 6 hours from 36 to 45°C	104
Temperature shift at 9 hours from 36 to 45°C	103
42°C (Fixed)	100

In Table A above, hydrolysis rate indicates glutamate accumulation rate, because glutamate is accumulated by hydrolysis of gluten. As is evident from these data, the "temperature shift" yields excellent hydrolysis rate. Specifically, the hydrolysis rate obtained with the claimed temperature shift results is superior to the rate obtained when the temperature is fixed at either 36 °C or 42 °C.

Also for the same temperature conditions above, the time course of glucose accumulation and consumption was monitored. The results are shown in Figure B below:

Figure B Time Course of Glucose Accumulation & Consumption



As shown in Figure B, the claimed temperature shift also yields execellent results in which residual glucose amount is kept low, contrary to degradation at 42°C.

Therefore, based on the foregoing, the Inventors of the '280 application successfully solved the problems associated with the commonly employed methods by shifting the temperature from lower temperature to higher temperature. Moreover, by adding the temperature shift into the process, the Inventors have achieved their goal of 1) maintaining hydrolysis speed and 2) hindering "browning reaction" due to the consumption of sugar. Nothing in the art of record would have led the artisan to this unexpected result.

12. I declare further that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

13. Further Declarant saith not.

Toshimasa Ishii

Toshimasa Ishii

April 21 /2003

Date